ORIGINAL RESEARCH ARTICLE

Association between 25-hydroxyvitamin D status and oral ovulation induction treatment outcomes in women with polyendocrine metabolic ovarian syndrome: A prospective cohort study, Western Cape, South Africa

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Abstract

This study determined the prevalence of suboptimal 25-hydroxyvitamin D (25-OH VD) levels in women with polyendocrine metabolic ovarian syndrome (PMOS), previously known as polycystic ovary syndrome (PCOS), in the Western Cape and assessed how 25-OH VD status affects oral ovulation induction treatment (OOIT) outcomes. In Phase 1, 29 participants completed questionnaires, underwent body composition assessment and provided blood samples for serum 25-OH VD. Phase 2 included 16 participants who underwent OOIT, with specialist feedback after one cycle. Suboptimal 25-OH VD levels occurred in 58.6% of participants. Follicle size increased by 1.1 mm per 10-unit rise in 25-OH VD (r=0.63, p=0.029). Vitamin D intake was inadequate in 96.6%, and 79.3% had a body mass index >24.9 kg/m^2. High body fat (>28%) was observed in 89.7% and insulin resistance in 72.0%. Among normal-weight participants, 57.1% had elevated body fat and insulin resistance. Suboptimal 25-OH VD levels were associated with smaller follicle size. *(Afr J Reprod Health 2026; 30 [22]: 15-27).*

Keywords: *:* Polyendocrine metabolic ovarian syndrome; polycystic ovary syndrome; vitamin D; ovulation; South Africa; fertility treatment.

Résumé

Cette étude a déterminé la prévalence de taux sous-optimaux de 25-hydroxyvitamine D (25-OH VD) chez des femmes atteintes d'un syndrome ovarien métabolique polyendocrinien (SOMP), auparavant appelé syndrome des ovaires polykystiques (SOPK), au Cap-Occidental, et a évalué l'influence du statut en 25-OH VD sur les résultats du traitement d'induction de l'ovulation par voie orale (TIOO). Lors de la phase 1, 29 participantes ont rempli des questionnaires, bénéficié d'une évaluation de la composition corporelle et fourni des échantillons sanguins pour le dosage de la 25-OH VD sérique. La phase 2 a inclus 16 participantes ayant reçu un TIOO, avec un retour du spécialiste après un cycle. Des taux sous-optimaux de 25-OH VD ont été relevés chez 58,6 % des participantes. La taille folliculaire augmentait de 1,1 mm par hausse de 10 unités de 25-OH VD (r=0,63, p=0,029). L'apport en vitamine D était insuffisant chez 96,6 %, et 79,3 % présentaient un indice de masse corporelle >24,9 kg/m^2. Une masse grasse élevée (>28 %) a été observée chez 89,7 % et une insulinorésistance chez 72,0 %. Parmi les participantes de poids normal, 57,1 % présentaient une masse grasse élevée et une insulinorésistance. Des taux sous-optimaux de 25-OH VD étaient associés à une taille folliculaire plus petite. *(Afr J Reprod Health 2026; 30 [22]: 15-27).*

Mots-clés: Syndrome ovarien métabolique polyendocrinien ; syndrome des ovaires polykystiques ; vitamine D ; ovulation ; Afrique du Sud ; traitement de la fertilité.

Introduction

Polyendocrine metabolic ovarian syndrome (PMOS), the recently adopted name for what was previously termed polycystic ovary syndrome (PCOS),[1] is a complex, heterogeneous endocrine and metabolic disorder that affects 8% to 13% of reproductive-aged women.[2] Women with PMOS often struggle to conceive due to multiple factors associated with the syndrome, including but not limited to hyperandrogenism and/or anovulation.[3] In addition to the latter, insulin resistance (IR) and

adiposity commonly coexist in women with PMOS, which further worsens hyperandrogenism in a multidirectional manner to negatively affect ovulation regularity and, consequently, the ability to conceive naturally.[4]

Hyperandrogenism exacerbates hyperinsulinaemia by impairing brain metabolism, leading to decreased leptin sensitivity, increased adiposity, impaired insulin signalling, increased IR in myofibres, and reduced insulin sensitivity.[4] Persistent hyperandrogenism disrupts the hypothalamic-pituitary feedback loop, halting oocyte maturation and resulting in multiple immature follicles, characteristic of subfertile anovulatory women with PMOS.[5,6]

Oral ovulation induction treatment (OOIT) with letrozole or clomiphene citrate is commonly used as the first-line fertility treatment in women with PMOS seeking pregnancy. However, 23% of women fail to respond to oral medications, with a 21% increased likelihood if deficient in 25-hydroxyvitamin D (25-OH VD).[7] Women with PMOS have significantly lower 25-OH VD levels compared to non-PMOS controls,[8-13] with an even greater likelihood in subsets with higher levels of adipose tissue.[12] Vitamin D (VD) plays a pivotal role in reproductive health by supporting both natural and induced ovulatory cycles. Adequate 25-OH VD levels enhance the granulosa cells' ability to support follicular growth and maturation through the production of steroid hormones, particularly oestrogen, which is essential during the follicular phase of the ovulatory cycle. 25-OH VD status influences the survival of developing follicles by inhibiting programmed cell death in granulosa cells and fostering a favourable follicular fluid environment, allowing follicles to mature and improving the chances of ovulation and, ultimately, conception.[14]

Rasheedy et al. explored VD supplementation as an adjuvant treatment for women with PMOS. The results of this study suggest that it may improve ovulation rates, enhance endometrial receptiveness, and modestly increase the likelihood of pregnancy.[15]

First-line treatment in PMOS is often used in combination with metformin, pioglitazone, or exenatide to target IR and hyperandrogenism in the PMOS population.[16] Women who do not respond to OOIT may proceed to second- or third-line treatments, including injectable gonadotrophins, laparoscopic ovarian drilling, or in vitro fertilisation (IVF) as a last resort.[17]

Strong evidence supports the maintenance of optimal 25-OH VD levels for healthy pregnancies and offspring health.[8,18-21] 25-OH VD screening is affordable, minimally invasive, safe, and sustainable in local healthcare settings, and could improve fertility treatment outcomes.[8,11,13,14,22-26] However, current fertility treatment protocols in South Africa do not include VD screening or supplementation. Further research is needed on VD intake from food and serum levels among South African women of reproductive age, considering the promising role VD has played in reproductive health. Current laboratory values for 25-OH VD focus on bone health rather than reproductive health, and more South African data may help warrant a review of current clinical guidelines to better support reproductive health and outcomes. This study aimed to determine 25-OH VD levels in women with PMOS residing in the Western Cape, and to assess how 25-OH VD status affects OOIT outcomes. Additionally, it sought to report on associations between 25-OH VD status, body composition, and VD food and supplement intake.

Methods

Study population, sampling and inclusion

This prospective cohort study recruited participants diagnosed with PMOS, aged 18–38 years and residing in the Western Cape province, through consecutive sampling over 17 months. The diagnosis of PMOS was made by the treating fertility specialists in accordance with the Rotterdam consensus criteria, which require the presence of at least two of the following three features after the exclusion of other aetiologies: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovarian morphology on ultrasound.[6] Recruitment occurred at a single fertility centre (Aevitas, Western Cape, South Africa) to minimise variation in diagnostic and treatment protocols. Participants were excluded if they presented with other causes of subfertility. A sample size of 90 was initially calculated with a biostatistician to enable logistic regression analysis of 25-OH VD levels on successful ovulation outcomes, aiming for 80% power and a 0.05 significance level. However, only 29 participants

were eligible for Phase 1, and only 16 proceeded to receive OOIT and were therefore included in Phase 2. The reduced sample size was due to the strict inclusion criteria, real-world recruitment challenges, and the time constraints of a master's project. Screening was performed by the research team, including the specialists, nurses, and the primary researcher.

Data collection: Phase 1

Participants completed a personal information questionnaire. Serum 25-OH VD levels were measured using a chemiluminescent microparticle immunoassay (CMIA) on the Abbott Architect instrument.[31] Body composition was analysed using an InBody-120 scale, and a validated VD food frequency and supplement questionnaire was completed and analysed using MRC FoodFinder together with manual supplement analysis. All participants were eligible for Phase 1.

Personal information questionnaire

Each participant completed a personal information sheet to describe the population and account for the covariables of 25-OH VD status and OOIT outcomes. Questions included age, fertility treatment history, skin covering practices, location, sun exposure, sunscreen use, Fitzpatrick skin type,[27] and medication use.

Blood sample to determine serum 25-hydroxyvitamin D

Serum 25-OH VD is the most reliable test for 25-OH VD status,[28] as it is the most abundant form, with a three-week half-life,[29] including both 25-hydroxycholecalciferol (25(OH)D3) and 25-hydroxyergocalciferol (25(OH)D2). Trained nurses drew 5 mL of blood by venepuncture into SST gel tubes. Samples were collected by Lancet-BARC Laboratories and transported under appropriate conditions. Analysis was performed using a CMIA on the Abbott Architect instrument.[29] Results remained blinded until requested after the observed treatment cycle and were then sent to the treating specialist. Unblinded results allowed the specialist to decide whether to improve 25-OH VD status before a subsequent cycle, which was excluded from analysis. To interpret 25-OH VD levels, laboratory cut-offs, relevant reproductive research, and recommendations from the Institute of Medicine (IOM) and the Endocrine Society were considered and classified as follows: deficient (<12 ng/mL), insufficient (12–20 ng/mL), sufficient (>20 ng/mL), and optimal (≥30 ng/mL).

Height measurement

A wall-mounted stadiometer with a sliding headpiece was validated using a metal rod of known length. Participants removed shoes, socks, and hair accessories. With the head in the midline, shoulders, buttocks, and heels against the wall, feet at 60 degrees outward, and arms relaxed, height was measured on an outward breath. The measurement was repeated three times, and the average was used.[33]

Body composition analysis with the InBody-120 device

Participants emptied their bladders and removed heavy clothing or accessories. Measurements were taken during follow-up appointments, at least three hours after exercise and two hours after oral intake. Researchers or trained nurses entered the participant code, height, and age. Barefoot, participants stood on the electrodes and held the handles with arms straight for a one-minute body composition test. The report included BMI, body fat percentage, visceral fat, and mineral levels.[33]

VD food frequency and supplement questionnaire

A 15-minute FFQ and supplement questionnaire was completed with the researcher's guidance. The questionnaire was previously developed and validated in a study on the determinants of 25-OH VD levels in healthy young adults in the Western Cape.[34] Section 1 included a five-page FFQ to quantify VD intake from South African foods; Section 2 was an open-ended question on supplement use. Participants reported weekly, daily, or monthly intake, with weekly being most common. All responses were converted to weekly values, analysed using MRC FoodFinder,[35] and then divided by seven for daily intake. Supplement data were manually analysed by reading product labels in stores. Supplement and food intakes were combined to calculate total daily VD intake.

Screening the need for metformin use

Participants were screened for metformin initiation per clinic protocol. Criteria included a BMI ≥30 kg/m², a history of OOIT resistance, or abnormal Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) or Quantitative Insulin-Sensitivity Check Index (QUICKI) scores, as noted in patient records.

Data collection: Phase 2

Participants who completed Phase 1 and proceeded to OOIT were included in Phase 2 after six weeks on metformin, if indicated. As per clinic protocol, letrozole was initiated on days 4–8 of the cycle. A sonogram was performed on day 12 or 13 to measure follicles, with follow-up scans every 3–4 days until follicles reached 16 mm. Daily blood tests followed to detect a luteinising hormone (LH) peak (>25 IU/L). Six days after the LH peak, ovulation was confirmed with a progesterone level ≥25 nmol/L. Specialists submitted feedback after one treatment cycle via a Microsoft form, automatically sent to the researcher.

Pilot study

Before data collection, a 60-day pilot study was conducted with five eligible participants. The researcher was supported by the Aevitas clinic team, including four specialists and five nurses. Training on participant identification, device use, and data collection procedures was provided, with checks to ensure compliance with standard operating procedures. Forms underwent face and content validity assessment by two peer master's students and a fertility clinic staff member. The full methodology was tested, with no changes required. Pilot study participants were included in the main cohort.

Outcome variables

The primary outcome variables were the prevalence of suboptimal serum 25-OH VD levels (classified from the venous blood sample using the cut-offs described above) and the response to OOIT, defined by follicle development and successful ovulation. Follicle size (mm) was measured by transvaginal sonography; the response to treatment was recorded as responder or non-responder; and ovulation was confirmed biochemically by a mid-luteal progesterone level ≥25 nmol/L following an LH peak >25 IU/L. The secondary outcome variables were total daily VD intake (IU) from food and supplements (quantified using the validated FFQ and supplement questionnaire), body composition (BMI [kg/m²], body fat percentage, and visceral fat level, measured using the InBody-120 device), glucose control and insulin resistance (fasting glucose, fasting insulin, HOMA-IR, and QUICKI), and mineral level (InBody-120). Covariables included age, season of blood sampling, Fitzpatrick skin type, sun exposure, sunscreen use, and medication use.

Data analysis

Stata 18 was used for analysis. Descriptive statistics (means, standard deviations, and medians for outliers) were calculated for continuous variables, and frequencies and percentages for categorical variables. The population's 25-OH VD status, body composition, glucose control, and VD intake from food and supplements were described.

A linear regression model adjusted for age assessed the relationship between serum 25-OH VD and follicle size. A p value <0.05 was considered statistically significant. Due to the short-term follow-up and small sample size, causal relationships could not be assessed, and the ability to detect statistically meaningful differences was limited. The findings were exploratory in nature.

Ethical considerations

The study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

Ethical approval was obtained from the Health Research Ethics Committee of Stellenbosch University (reference number S22/10/222). Written informed consent was obtained from all participants prior to enrolment, and participation was voluntary and confidential. Participants were free to withdraw at any stage without affecting their clinical care.

Results

Demographics and clinical characteristics of participants

A cohort of 29 participants residing in the Western Cape and diagnosed with PMOS was included in the study.

Table 1: Serum 25-hydroxyvitamin D levels of women with PMOS residing in the Western Cape

Category	Reference range	Vitamin D blood results n (%); n=29
Deficient for bone health in the general healthy population	<12 ng/mL	1 (3.4)
Insufficient for bone health in the general healthy population	12–20 ng/mL	7 (24.1)
Sufficient for bone health in the general healthy population	>20 ng/mL	21 (72.4)
Possibly optimal for reproductive health	≥30 ng/mL	12 (41.4)
Possibly suboptimal for reproductive health	<30 ng/mL	17 (58.6) (95% CI: 40.7–74.5)

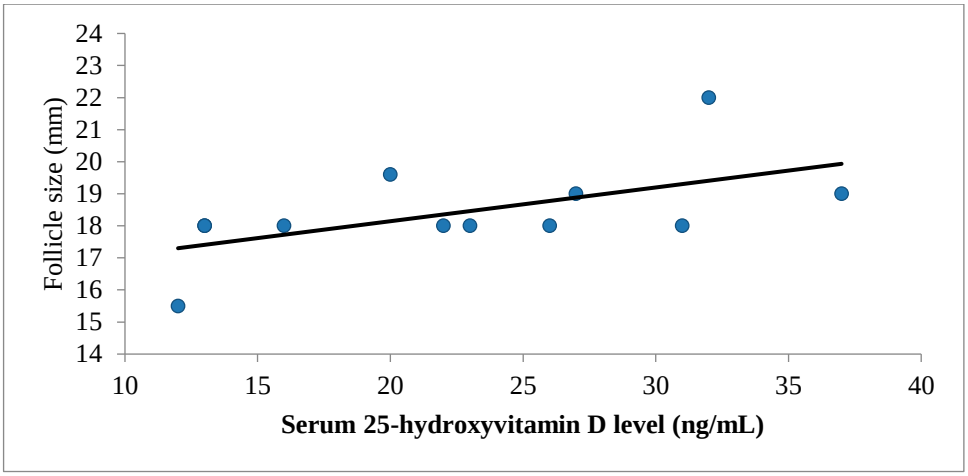

Figure 1: Association between serum 25-hydroxyvitamin D level (ng/mL) and follicle size (mm) for the 12 participants who developed at least one follicle during oral ovulation induction treatment. The solid line represents the linear regression fit (r=0.63, p=0.029). For participants who developed more than one follicle, the largest follicle is shown.

The mean age of the cohort was 31.3 ± 4.2 years, with 75.0% of individuals aged between 29 and 34 years (range: 21–38 years).

Skin type was determined using the Fitzpatrick scale to allow for analysis of potential associations with 25-OH VD levels.

Primary objectives

The prevalence of suboptimal serum 25-hydroxyvitamin D levels

Among the cohort, 58.6% (n=17) of participants had suboptimal 25-OH VD levels for reproductive health (<30 ng/mL).

Within this group, 27.6% (n=8) were further categorised as having insufficient levels (<20 ng/mL) or deficient levels (<12 ng/mL).

Of the 29 blood samples, 20 (69.0%) were tested during the summer months, while 9 (31.0%) were obtained during the winter months. Table 1 details the results.

Response to OOIT in the PMOS population

From the initial cohort, 16 participants proceeded to receive OOIT. Of these 16 participants, four (25.0%) were non-responders and 12 (75.0%) responded to treatment, but only nine ovulated successfully. A bi-follicular response was observed in four (25.0%) participants. The responder and non-responder groups were too small for statistical analysis. The median follicle size was 18.0 mm, with an interquartile range (IQR) of 1.0 mm. A moderate positive correlation existed between 25-OH VD levels and follicle size (r=0.63, p=0.029). For every one-unit increase in 25-OH VD, follicle size increased by 0.11 mm (95% CI: 0.004–0.22 mm, p=0.044, adjusted for age). Thus, for every 10-unit increase in 25-OH VD, follicle size increased by 1.1 mm on average. Figure 1 presents the association between serum 25-OH VD levels and follicle size for the 12 participants who developed at least one follicle during OOIT, together with the fitted regression line.

Table 2: Blood glucose control in PMOS participants

Variable	Value
Fasting glucose level (mmol/L)	5.0 ± 0.4 (mean ± SD)
Fasting insulin (IU/L)	12.1 (7.2) (median, IQR)
Insulin resistance (HOMA-IR[a]/QUICKI[b])	n=18/25 (72.0)

[a] Homeostatic Model Assessment of Insulin Resistance; [b] Quantitative Insulin-Sensitivity Check Index

Table 3: Ability of body mass index versus fat percentage to predict the likelihood of insulin resistance

BMI category	n (%); n=25	Confirmed insulin resistance (HOMA-IR/QUICKI)	Fat % out of range (>28%)
Obese	8/25 (32.0)	8/8 (100.0)	8/8 (100.0)
Overweight	9/25 (36.0)	7/9 (77.8)	9/9 (100.0)
Normal	7/25 (28.0)	4/7 (57.1)	5/7 (71.4)
Underweight	1/25 (4.0)	0/1 (0.0)	0/1 (0.0)

Secondary objectives

Vitamin D intake

The recommended dietary intake (RDI) for VD is set at 600 IU.[36] In this study, 96.6% (n=28) of the cohort did not meet this recommendation from food alone. The median intake from food was 172.0 IU (IQR: 138.0–202.0 IU), representing the central tendency due to outliers. In this cohort, 48.3% (n=14) used cholecalciferol (VD3) supplements, with doses ranging between 400 IU and 4 663 IU. The median intake among those who used supplements was 400.0 IU (IQR: 0–1 000 IU), underscoring the high variability in supplementation levels. The median intake of VD from food and supplements was 278.0 IU (IQR: 138.0–700.0 IU), indicating that 50% of the sample achieved less than half (47.0%) of the RDI. No significant association was identified between 25-OH VD levels and sunlight exposure (ρ=0.16, p=0.515).

Body composition

BMI was above normal in 79.3% (n=23) of the participants, with 37.9% in the overweight category (BMI 24.9–29.9 kg/m²) and 41.4% in the obese category (BMI ≥30 kg/m²). Additionally, 89.7% (n=26) of the cohort presented with a fat percentage above the normal range. The normal fat percentage range on the InBody software of 18–28% was determined from various adult study populations. The mean visceral fat level was above the normal range (>9) in 82.8% of the cohort. The association between 25-OH VD levels and body composition was not statistically significant for BMI (ρ=−0.22, p=0.259) or visceral fat level (ρ=−0.27, p=0.155).

25-Hydroxyvitamin D level categories and mineral levels

Participants were grouped by 25-OH VD level (deficient, insufficient, sufficient, and optimal). Mineral levels varied slightly across these groups. The mineral level measured by the InBody-120 scale serves as a proxy for bone mineral content and overall skeletal health. The InBody scale provides mineral reference ranges based on population data. The mineral level represents the total amount of inorganic minerals (primarily from bone and body fluids) and is adjusted for age, sex, and body weight. The overall mean mineral level across all categories was 3.3 ± 0.5. A weak positive but non-significant correlation was observed between 25-OH VD and mineral levels (Spearman's ρ=0.14, p=0.459).

Insulin resistance in women with PMOS

Table 2 summarises the blood glucose control recorded in participants.

The association between serum 25-OH VD and QUICKI was not statistically significant (ρ=0.22, p=0.292); however, a positive trend was observed. IR was confirmed with a HOMA-IR or QUICKI test in 72.0% (n=18) of the cohort. Among the seven participants with a normal BMI who were screened for IR, four (57.1%) presented with insulin resistance and three (42.9%) did not. Of the 29 participants included in Phase 1 of the study, 25 underwent screening for IR; the remaining four had financial constraints and could not complete the HOMA-IR/QUICKI tests. Of the 16 participants included in Phase 2, one participant was not tested for IR, bringing the total down to 15.

Discussion

This prospective cohort study is, to the best of our knowledge, among the first to examine the association between serum 25-OH VD status and OOIT outcomes in South African women with PMOS, and its findings are best understood within the context of the wider international literature. Suboptimal 25-OH VD status is common in populations worldwide,[32] and, globally, around 60% of the PMOS population has suboptimal 25-OH VD levels. This study found comparable results, with 58.6% of the cohort presenting with 25-OH VD levels <30 ng/mL. However, a degree of uncertainty exists owing to the wide confidence interval (40.7% to 74.5%) and the study's small sample size, which limited the ability to detect significant associations. Consequently, only trends could be observed in this analysis. Among the variables examined, sun exposure correlated the least (ρ=0.16, p=0.515) with 25-OH VD levels, compared with IR (ρ=0.22, p=0.292), BMI (ρ=−0.22, p=0.259), and visceral fat level (ρ=−0.27, p=0.155). Additionally, 65.5% of participants used sunscreen regularly, further reducing cutaneous VD synthesis. Despite high rates of sun protection and limited exposure, the expected association was not observed in this study, nor has it been consistently demonstrated elsewhere, owing to variability in sunscreen application, formulation, and user behaviour.[37,38] Public health authorities, including the Cancer Association of South Africa and the World Health Organization, advise against excessive sun exposure and recommend protective strategies such as the use of hats, clothing, shade, and sunscreen. From a physiological standpoint, sunscreens that block up to 100% of ultraviolet B (UVB) radiation inhibit cutaneous VD production, which could theoretically lead to lower serum 25-OH VD levels.

However, real-world evidence shows variation in outcomes. Even in regions with abundant year-round sunlight, fluctuating UVB radiation influences the conversion of VD during seasonal changes. This may explain the variability observed in serum 25-OH VD levels across seasons, with summer months yielding higher levels than winter months, and peak synthesis occurring around midday during periods of optimal exposure.[39,40,41] Individuals with PMOS remain at risk of suboptimal VD levels despite sunny geographical locations and favourable latitudes. This susceptibility may be attributed not only to behavioural and environmental factors, but also to the complex metabolic profile associated with PMOS, which includes IR, elevated BMI, and increased visceral fat, all of which may further compromise VD status.[25] Seasonal variation may warrant more frequent screening by specialists, particularly during winter. Clinicians should prioritise patient education on maintaining optimal 25-OH VD levels through diet, supplementation, and responsible sun exposure to mitigate seasonal fluctuations and support reproductive health.

25-Hydroxyvitamin D and OOIT outcomes in women with PMOS

25-OH VD levels were significantly associated with follicle size. For every 10-unit increase in 25-OH VD level, follicle size increased by 1.1 mm on average. An increase in follicle size, particularly when 15 mm or larger, is associated with an increased likelihood of successful ovulation and, ultimately, pregnancy. The impact of VD on reproductive health in this population is multidimensional. One important mechanism is its support of follicular development, which plays an important role in follicular growth. VD supports these granulosa cells in producing oestradiol, progesterone, and follicle-stimulating hormone. Additionally, VD supports the survival of developing follicles by inhibiting programmed cell death of the granulosa cells. It also supports a favourable follicular fluid environment in which follicles can thrive, which ultimately leads to ovulation.[42]

Vitamin D intake from food and 25-hydroxyvitamin D status in South African women with PMOS

None of the participants were able to meet their RDI for VD through diet alone. The absorption of VD occurs in the small intestine and is facilitated by the presence of dietary fat. The South African diet is not typically abundant in fatty fish, egg yolks, or liver. Dietary VD can be obtained through either VD3 from animal products or ergocalciferol (VD2) from plant foods. Vitamin D3 exhibits higher bioavailability compared to VD2. Currently, no studies have been conducted in the PMOS population in South Africa that specifically report on VD intake from food and the corresponding 25-OH

VD serum levels. However, the findings align with the limited data on South African VD food intake, which reports an intake of around 120 IU per day—significantly below the RDI of 600 IU. While dietary VD intake contributes to overall VD status, it is not an independent predictor because it is influenced by multiple factors, including urbanisation, lifestyle, disease status, ethnicity, and body composition. Rather than acting independently, these factors likely interact to collectively determine circulating 25-OH VD concentrations.[34] Together with a limited variety of VD-fortified food products and urbanisation, this may help explain the gap between RDI and intake.[34]

Vitamin D intake from supplements and 25-hydroxyvitamin D status in women with PMOS

The association between VD supplementation and 25-OH VD status was not significant in this study. Supplementation did, however, show the strongest association when analyses were conducted between 25-OH VD levels and supplementation dose, sun exposure, VD intake from food, and body composition. This finding is consistent with other studies in which VD supplementation can increase serum 25-OH VD levels, but the response is often blunted, particularly in women with higher levels of adiposity, which is common in PMOS[22,43] and in this study population. This outcome also mirrors findings in broader populations, where body weight and adiposity are recognised as key factors influencing VD, which is often sequestered in adipose tissue, reducing its availability in circulation.[22] These findings suggest that, while VD supplementation is beneficial, a more personalised approach is needed—taking into account the combined impact of variable factors in PMOS, such as body composition and IR, to optimise 25-OH VD levels.[13,22,26,42,43]

Mineral level and 25-hydroxyvitamin D levels

The weak positive correlation between 25-OH VD levels and mineral levels observed in this study may be explained by the fact that VD plays a multifaceted role in mineral homeostasis. VD increases the absorption of calcium and phosphate, which are essential for bone mineralisation and, ultimately, for maintaining a balance between various mineral levels. However, this relationship is affected by factors such as genetics, diet, and renal function, which could change mineral concentrations independently of 25-OH VD status. This complex relationship explains the weak correlation observed in this study.[29,30]

Body composition in PMOS

In this study, the largest proportion of participants were either obese or overweight, with a high prevalence of both total body fat and visceral fat. This aligns with the findings of the systematic review and meta-analysis by Zhu et al., which reported that 58.5% of the PMOS population presented with abnormally elevated body fat percentages compared with their BMI-matched controls (49.3%). Additionally, women with PMOS have been found to have, on average, 0.31–0.47 standard deviations higher amounts of visceral fat compared with the general female population.[44,45] IR and hyperandrogenism, typically present in the PMOS population, increase the likelihood of elevated adipose tissue, particularly in the abdominal region, making the PMOS population more prone to higher fat levels.[45] Overweight and obesity rates in the South African population are estimated at 60% and 35.2%, respectively.[46] In this cohort, the reported overweight and obesity rates were 37.9% and 41.4%, respectively. However, it should be noted that the body composition data revealed high body fat percentages and visceral fat levels, irrespective of BMI.

Body composition and OOIT outcomes in women with PMOS

Body composition showed no significant associations with OOIT outcomes in this study, likely due to the small sample size. However, larger studies have reported contrasting findings, indicating that body composition—particularly body fat percentage—affects the outcome of OOIT in women with PMOS.[7,22,44] While participants were not subclassified according to their phenotype in this study, considering the high prevalence of adiposity, it may be reasonable to assume that the majority in this cohort would likely have been classified as phenotype A. Type A is associated with higher body fat, which may impact fertility.[47] In women diagnosed with PMOS, body composition may be a strong influential factor for fertility; however, it is not the only one. Although high body weight and/or fat percentages can exacerbate hormonal imbalance, normal-weight women with PMOS may still present with ovulatory dysfunction, as seen in the phenotype

D subgroup. Genetics, chronic inflammation, and other metabolic irregularities may also contribute to ovulatory function, independent of body composition.[47]

Insulin resistance and 25-hydroxyvitamin D levels

Although not significant, QUICKI showed a positive correlation with 25-OH VD levels. The relationship between 25-OH VD status and IR is bidirectional. 25-OH VD status positively impacts insulin sensitivity, and higher insulin sensitivity is associated with higher QUICKI results, which in turn correlate with higher 25-OH VD levels, likely due to improved bioavailability.[3,8,25,43]

Insulin resistance and OOIT outcomes in women with PMOS

IR also showed insignificant associations with OOIT outcomes. It commonly coexists with a PMOS diagnosis, with or without obesity, in 70% of women with this condition. These findings are similar to those in this study, where 57.1% (4/7) of normal-weight individuals presented with raised adiposity and IR. Elevated insulin levels exacerbate rising testosterone levels and further decrease sex hormone-binding globulin (SHBG), which in turn raises free testosterone levels, increasing fat storage and further worsening IR. These metabolic and hormonal imbalances may, in part, explain the cycle irregularities observed in this population.[29] Furthermore, hyperandrogenism is associated with a high body fat percentage, which increases hyperinsulinaemia, decreases leptin sensitivity (further increasing adiposity), and results in impaired insulin signalling, exacerbating IR.[41] This mechanism blunts the hypothalamic-pituitary feedback loop, raising LH secretion, causing premature granulosa cell luteinisation, and ultimately halting oocyte maturation.[43] Therefore, identifying and treating IR through interventions such as metformin and/or lifestyle changes can significantly improve OOIT outcomes, as treating IR enhances insulin sensitivity and restores ovulatory function. The high prevalence of IR, obesity, and adiposity in this study aligns with findings from other studies on PMOS, where metabolic complications are common. The results of this study reiterate the importance of using body composition—particularly visceral fat levels—rather than BMI in isolation, to identify patients at risk of metabolic complications, such as IR, which can impact fertility. This is particularly relevant where more expensive screening tests, such as HOMA-IR or QUICKI, are not available. If BMI had been used as a screening tool, IR would have been missed in 57.1% of the participants. Further, the notion of subclassifying women with PMOS based on their phenotypes is essential for guiding intervention strategies, as targeted phenotypic interventions address the underlying cause of subfertility.[3]

Body composition and VD status in women with PMOS

In this study, while 25-OH VD levels correlated negatively with both BMI and visceral fat levels, these correlations were not statistically significant. This is likely due to the small sample size, although the trends are consistent with previous research.[11,48] The rationale for an association between serum 25-OH VD levels and body composition in this population can be explained by the sequestration of VD in fat tissue, which results in a reduction in circulating serum 25-OH VD. Up to 30% of the normal-weight individuals diagnosed with PMOS still presented with elevated visceral fat levels.[44,45,48]

Strengths and limitations

The principal strengths of this study lie in its prospective cohort design, conducted in a real-world clinical setting, which allowed serum 25-OH VD status to be linked directly to objectively measured ovulation induction outcomes in an understudied South African population. The combined use of a validated VD food frequency and supplement questionnaire, a standardised immunoassay for serum 25-OH VD, and direct body composition analysis (rather than BMI alone), together with the inclusion of seasonal sampling and Fitzpatrick skin typing, strengthens the validity of the findings.

Several limitations should, however, be acknowledged. The small sample size limited the statistical power to detect significant results, and the intended sample size was not reached owing to the strict inclusion criteria and a range of real-world factors. These included other ovulatory-related causes of subfertility, the single geographical location, loss to follow-up, financial constraints,

poor compliance with metformin, and pregnancy occurring during the window of metformin intervention—all of which affected participant retention. In addition, the MRC FoodFinder database remains incomplete, as not all food items have been analysed for their VD content, and the nutrient composition of produce varies across the different geographical settings of South Africa. The accuracy of the dietary information obtained from the FFQ may also have been affected by recall bias. The follow-up window was short, as only a single treatment cycle was observed; this may not be a true reflection of the response across multiple cycles, as the same individual may respond differently in subsequent cycles. Finally, long-term outcomes, such as confirmed pregnancies and live births, were not explored in this short-term cohort. These limitations should be addressed in future, adequately powered, multicentre studies.

Implications for policy and practice

Despite these limitations, the findings carry several implications for clinical practice and policy. The high prevalence of suboptimal 25-OH VD levels, inadequate dietary VD intake, and insulin resistance—even among normal-weight women—supports the routine assessment of vitamin D status and body composition, rather than BMI alone, in the management of women with PMOS who are seeking fertility treatment. Given that 25-OH VD screening is affordable, minimally invasive, and widely available, its integration into local fertility protocols, together with individualised supplementation and patient education on diet, supplementation, and responsible sun exposure, may offer a low-cost strategy to support reproductive outcomes. At a population level, the consistently low dietary VD intake observed reinforces the need to consider food fortification strategies in South Africa. These findings should inform the design of larger, adequately powered studies to confirm the role of vitamin D status in ovulation induction and to guide the future revision of clinical guidelines.

Conclusion

This Cape Town-based study makes a valuable contribution to understanding the suboptimal 25-OH VD level trend observed globally in women with PMOS. It reiterates the crucial physiological role of VD in promoting follicular maturation, supporting regular ovulatory cycles, and ultimately increasing the chance of successful conception. The findings align with global results and highlight the substantial proportion of women with PMOS who have elevated body fat percentages and visceral fat levels, which contribute to a higher prevalence of metabolic imbalances.[44,45,49] Notably, IR was present in 72.0% of the cohort, consistent with global data indicating that 50–80% of women with PMOS experience IR, regardless of BMI.[47] This underlines that BMI alone may underestimate metabolic risks, as participants with a BMI within the normal range exhibited elevated body fat percentages (57.1%, n=4/7). These findings support the notion of using body composition instead of BMI alone to determine metabolic risks, such as increased adiposity and IR, which could affect fertility outcomes. Suboptimal 25-OH VD levels are not well documented in the general South African population. However, the PMOS population faces a unique challenge due to the combined influence of variables such as IR, adiposity, and hormonal imbalances on 25-OH VD levels. The findings from this study reiterate the complexity of PMOS and its intricate connections with IR, VD, body composition, and hyperandrogenism in relation to ovulatory success. To improve VD status in this population, screening with individualised and targeted supplementation regimes is needed, together with fortification strategies for general food products in South Africa.

Data availability statement

The data that support the findings of this study and the related study tools are available from the corresponding author (Lindie Mosehuus) upon reasonable request.

Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Contribution of authors

Lindie Mosehuus conceived and designed the study, coordinated participant recruitment and data collection, performed the data analysis and interpretation, and drafted and revised the manuscript. Zarina Ebrahim and Janicke Visser supervised the study, contributed to its conceptualisation and methodology, and critically reviewed and edited the manuscript. Thomas I. Siebert provided clinical oversight of participant diagnosis and ovulation induction treatment, contributed to the study design, and critically reviewed the manuscript. Carl J. Lombard provided statistical guidance, contributed to the design of the analysis and the interpretation of the results, and critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

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